ENFORCEMENT ANALYTICAL METHOD FOR THE DETERMINATION OF OCTANOIC ACID BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

1. SUMMARY

This method is validated for the determination of octanoic acid in Reducx. This report is intended to satisfy US EPA Product Properties Testing Guideline OPPTS 830.1800. The method validation was performed in conjunction with accelerated storage stability and corrosion characteristics testing of Reducx, as per US EPA Product Properties Testing Guideline OPPTS 830.6317 and 830.6320 (reference PSL Study Number 49074). This study was conducted in compliance with 40 CFR Part 160: U.S. EPA (FIFRA).

2. APPARATUS

High Performance Liquid Chromatography (HPLC) System: Agilent 1100 with DAD or equivalent
Column: Agilent Eclipse XDB-C18, 4.6 x 250 mm, or equivalent, 5µm,

3. CHROMATOGRAPHIC PARAMETERS

See Table 1 for details

4. PROCEDURE FOR THE DETERMINATION OF OCTANOIC ACID BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

A. Reference Standard(s)

Name: Octanoic acid
Batch No.: BCBS8687V
Purity: 99.8%
Expiration Date: September 2019
Supplier: Sigma-Aldrich

B. Chemical Analysis

4.B.1 Standard Preparation:

Linearity Solution Preparation: Prepare six linearity solutions, as shown below, by accurately weighing appropriate amounts of reference standard into suitably sized volumetric flasks. Add diluent to volume (15 grams erythorbic acid + 250 mL acetonitrile + 250 mL deionized water), and mix well. Sonicate solutions for ~20 minutes.

Standard ID	Weight (g)	Final Volume (mL)	Expected Concentration (mg/mL)
Lin 1	0.0061	50	0.1218
Lin 2	0.0048	20	0.2395
Lin 3	0.0069	20	0.3443
Lin 4	0.0110	20	0.5489
Lin 5	0.0079	10	0.7884
Lin 6	0.0102	10	1.0180

4.B.2 Test Substance and Spike Sample Preparation:

Sample Preparation: Prepare replicate sample solutions by accurately weighing approximately ~0.2 grams of the test substance into separate 20 mL volumetric flasks and bringing to volume with diluent. Stopper and sonicate solutions for ~30 minutes. Allow to equilibrate to room temperature and then mix well.

Accuracy Solution Preparation: Prepare spike sample solutions at two different fortification levels, (designated as Low and High Spike) by accurately weighing and transferring appropriate amounts of the thoroughly mixed test substance and reference standard into separate 20 mL volumetric flasks, adding diluent to volume, and mixing well. Allow sample solutions to cool to room temperature and then mix well.

Sample ID	Sample ID	Weight Used (g)	Final Volume (mL)	Expected Conc. (mg/mL)
Low Spike	Test Substance	0.0663	20	0.3796
	Standard	0.0042		
High Spike	Test Substance	0.0881	20	0.7200
	Standard	0.0099		

4.B.3 Method Validation:

Linearity: Perform a single injection of each linearity solution, as described in Section 4.B.1 (c) using the instrument conditions in Table 1. Record the peak area responses using the chromatography software. Perform a linear regression analysis of the resultant peak area response as a function of the standard solution concentration. A correlation coefficient of ≥ 0.995 is considered acceptable.

Precision: Perform analysis of five preparations of the test substance, as described in Section 4.B.2 (d). Determine the relative standard deviation (RSD) of the active ingredient concentration. An RSD of $\leq$ 2% is considered acceptable.

Accuracy: Perform duplicate injections of the spike solutions, as described in 4.B.2 (d). Calculate the amount of active ingredient (AI) recovered in the spike samples and compare it to expected amount. A mean percent recovery of 98-102 % is considered acceptable.

4.B.4 Analysis:

Filter aliquots of each solution through a 0.45µm syringe filter, and then transfer to auto-sampler vials for analysis. Prior to analysis, equilibrated the instrument until stable operating conditions are attained, using instrument conditions described in Table 1. Prepare an analysis sequence containing solvent blanks, formulation blanks, standards, and test substance. Record the peak area responses. Calculate the amount of active ingredients found as shown below.

4.B.5 Calculations:

Perform all calculations using Excel (or equivalent) with full precision. Minor differences may be found between the values reported and those obtained if calculated manually.

% AI = Calc. AI Conc. (mg/mL)/Sample Conc. (mg/mL) * 100

Where:

Calc. AI Conc. (mg/mL) = Peak Area / Average Response Factor

Response Factor = Peak Area / Standard Conc. (mg/mL)

Sample Conc. (mg/mL) = Sample Weight (g) / Sample Volume (mL) *Sample Dilution * 1000 (mg/g)

Standard Conc. (mg/mL) = Standard Weight (g) / Standard Volume (mL) *Standard Dilution * Standard Purity (%)/100 *1000 (mg/g)

C. Method Validation Results

Validation of this method was performed under 40 CFR Part 160: U.S. EPA (FIFRA) Good Laboratory Practices in Product Safety Labs Study Number 49134. HPLC operating conditions are recorded in Table 1. Linearity results are presented in Table 2. Method Validation Results of test sample analyses are presented in Table 3. Representative chromatograms are presented in Appendix A. The Certificate of Analysis is presented in Appendix B.

A summary of the method criteria and results are presented below.

Octanoic Acid			
Parameter Evaluated	**Acceptance Criteria**	**Results**	**Acceptable (Yes/No)**
Linearity Range: 0.1218-1.0180 (mg/mL)	$r \geq 0.995$	0.999	Yes
Accuracy	98-102%	100.84%	Yes
Precision	RSD $\leq$ 2%	0.78%	Yes

SIGNATURE

Reducx

I, the undersigned, declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

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Date